STUDIES ON THE STRUCTURE OF LIPOPROTEIN A OF HUMAN HIGH DENSITY LIPOPROTEIN HDL_3: THE SPHERICALLY AVERAGED ELECTRON DENSITY DISTRIBUTION

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1. Introduction

In a previous article [1] we gave a first report on the experimental results obtained by X-ray small angle scattering of the LpA fraction of human plasma high density lipoprotein HDL_3 in 0.15 M NaCl solution. The shape of the scattering curve showing three characteristic side maxima reflected a high structural regularity of the molecule.

In the present paper we give a more detailed analysis of the scattering curve mentioned above, as well as additional results from X-ray small angle scattering experiments in 36% sucrose solution. The results are strongly in favour of a spherical molecular model of 96 Å diameter consisting of an inner part of low electron density surrounded by a high electron density shell.

2. Materials and methods

2.1. *Sampling*

The isolation and purification procedure of the LpA fraction from human HDL_3 was according to the methods of Kostner and Alaupovic described earlier [2, 3]. For the experiments in sucrose solution the preparations were exhaustively dialized against a solution containing 0.15 M NaCl and 36% (w/w) sucrose.

All solutions containing 0.05% (w/v) Na_2EDTA and NaN_3 respectively. Sample concentrations were determined gravimetrically from the dry weight.

2.2. *X-Ray small angle measurements*

The experimental details were essentially the same as described previously [4, 5]. All measurements were performed at a constant sample temperature of 4°C using an electronically controlled cuvette [6]. The experimental scattering curves were corrected for the influence of the K_β-line [7] and for collimation effects due to the line shaped primary beam [8] using an iterative mathematical procedure [9, 10]. A constant background accounting for the 'liquid structure' within the particles was subtracted from the scattering curves afflicted with the collimation error [5, 11]. The inner parts of the scattering curves were measured at different sample dilutions and extrapolated to zero concentration. All computer calculations were performed at a UNIVAC 494 of the Rechenzentrum Graz.

3. Results and analysis

As demonstrated in fig. 1 the scattering curve of

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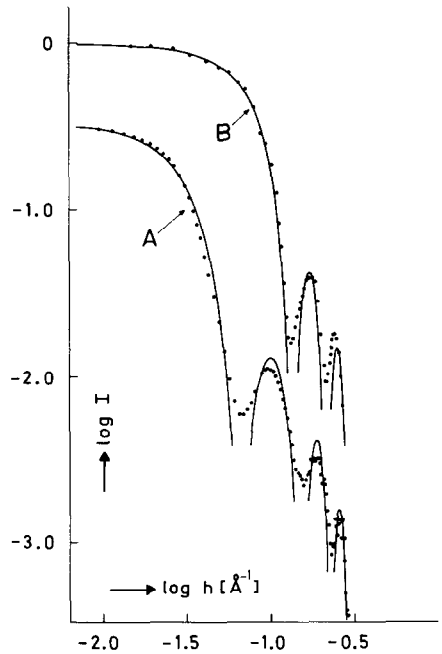

Fig. 1. Experimental scattering curves of LpA in 0.15 M NaCl solution containing 0% (A) and 36% (B) sucrose. Heavy full lines show the theoretical scattering curves of the models demonstrated in fig. 2. The scaling of the ordinate is arbitrary.

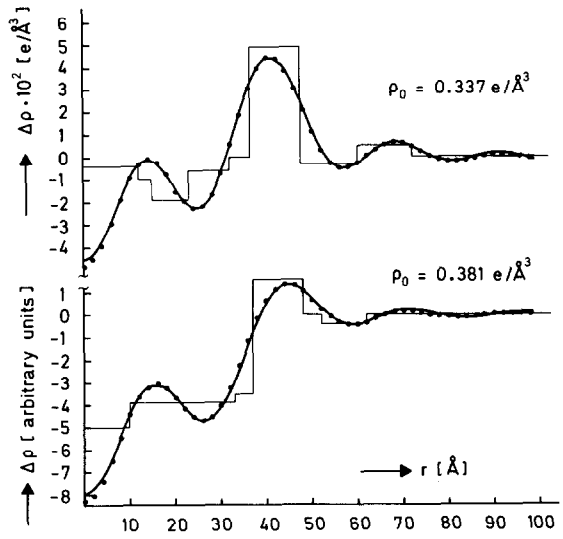

Fig. 2. Fourier transforms of the structural amplitudes in 0% (ρ_{el} = 0.337 e/Å^3) and in 36% sucrose (ρ_{el} = 0.381 e/Å^3) solution (heavy fulllines). The dots show the theoretical Fourier transforms of the step models (light full lines).

$$\rho_{(r)} \cdot r = \frac{K}{2\pi^2} \cdot \int_0^\infty F_{(h)} \cdot h^2 \cdot \frac{\sin hr}{hr} \cdot dh$$

LpA in 36% sucrose solution shows two distinct side maxima which are displaced with regard to their angular position as compared to the maxima obtained in solution without sucrose. The radius of gyration determined from the inner part of the scattering curve according to Guinier and Fournet [12] in 36% sucrose solution is 18.1 Å, which is also at variance with the value of 50.5 Å found in 0.15 M NaCl solution.

In order to explain these results in terms of the molecular structure a series of theoretical scattering curves were calculated assuming models of various size and shape including subunit models similar to those arising from the interpretation of electron micrographs by Forte et al. [13, 14]. None of these curves did in any respect show convincing similarities to our experimental curves. In the course of these calculations the assumption of a spherical structure became increasingly probable.

For spehrical particles the radial electron density distribution can be obtained by the Fourier transformation of the structural amplitude F(h):

This treatment requires the extrapolation of the scattered intensity to zero in the region of the minima, since especially there the scattering curve is most sensitive to minor deviations from perfect spherical symmetry of the molecule. A manageable amplitude curve was obtained by plotting the square root of the experimental curves positively and negatively and connecting the maxima by smooth lines crossing the abscissa [15]. The amplitude bands were given alternating signs starting with a positive first maximum.

The results of the Fourier transformations at 0% and 36% sucrose concentration (ρ_{el} = 0.337 e/Å^3 and 0.381 e/Å^3, respectively) are shown in fig. 1. In both cases the termination effect corresponds to Bragg's angles of 23 Å. An absolute scale determination of the distribution function in terms of e/Å^3 was performed for the measurements without sucrose only, since uncertainties in the sample concentration in the sucrose solutions prevented the exact evaluation of the constant K in the above equation. However, the characteristics of the two curves exhibit striking similarities.

Since these distribution functions are afflicted with

the termination effect they do not directly reflect the real electron density distribution. Therefore we calculated the Fourier transforms of a series of step models terminating the theoretical amplitude curves at the same angle as the experimental ones. The step functions which give a satisfactory fit to the experimental Fourier transforms were found by trial and error. These are shown in fig. 2. Obviously the step functions are only approximative representations of the real electron density distribution, for the mere reason that the assumption of a step function is certainly not holding exactly for the real molecule. Furthermore the deviations of the step functions from the real but unknown distribution functions are reflected by the differences between the experimental and theoretical scattering curves, which are shown in fig. 1. Of course, all experimental errors influence the found step functions. All these facts might also explain the differences between the two model functions at radii lower than 20 Å.

4. Discussion

The analysis of the experimental results show that the overall shape of LpA does not diverge strongly from spherical symmetry. The radial electron density distribution represents a spherical average of the internal composition of the molecule. Obviously the step corresponding to this distribution only gives a highly idealized picture of the real structure. However, the results allow several important conclusions on the structure of LpA.

The most significance result is the high electron density region near the radius of 40 Å which indicates that this part of the molecule contains the polar head groups of the phospholipids and probably most of the constituent peptides. This gives a direct structural explanation for the observation that both the ester bonds of the phospholipids and the polypeptides are readily accessible to chemical and enzymatical action [16, 17]. Despite the fact that the two constituent polypeptide classes, ApoA-I and ApoA-II represent different structural entities [18], they must be similarly distributed at the molecular surface closely interacting with the polar groups of the phospholipids.

Another striking fact is the radius of about 37 Å of the inner electron deficient region. Even fully stretched hydrocarbon chains could not account for this dimension. A possible explanation could be provided by the assumption of an extended conformation of the cholesterol esters which by a radial arrangement together with the remnant hydrocarbon chains of the phospholipids and triglycerides form a spherical micellar structure. This would be consistent with the interpretation of NMR and ESR experiments on high density lipoproteins [19, 20]. Although the information on the electron density close to the center of the molecule most be regarded with precaution, the assumption of a protein or other high electron density core is very unlikely. Since the electron density at lower radii than 37Å does not drop immediately to low values, an interaction of polypeptide chains with the lipid hydrocarbon chains is indicated.

The electron density fluctuations beyond 48 Å can be ascribed to both the deviations from perfect spherical shape and salt effects due to the high surface charge of the molecule.

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